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Entrainment of Tidal and Semilunar Rhythms by Artificial Moonlight Cycles

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Abstract. A marked feature of the larval release activity of the terrestrial crab *Sesarma haematocheir* is its synchronization with the time of high water. This activity occurs only at night, so that the pattern of the tidal rhythm recurs at semi-monthly intervals. When adult specimens from Seto (Okayama Prefecture) and Shima (Mie Prefecture) populations were brought from the field into 24-h light-dark conditions in spring, the larval release occurred at night, but the overall activity pattern gave no indication of a tidal component. On the contrary, under simulated moonlight cycles the timing of release was strongly coordinated and exhibited a well-defined tidal component arranged at semi-monthly intervals. The phase difference between the evoked tidal rhythms of the two populations of 4–5 h was about equal to the phase difference of the tidal cycles in their natural habitats. Synchronization of larval release with the artificial moonlight cycle required more than 40 days of exposure. In addition to entraining the tidal rhythm, artificial moonlight induced a semilunar rhythm in both populations. Entrainment could be achieved with exposure to moonlight for just a few days around the time of the full moon. In this paper, underlying mechanisms of the *Sesarma* larval release rhythm, which involves both tidal and semilunar components, are explained in terms of circadian oscillatory systems.

Introduction

From the viewpoint of ecological adaptation of biological timing systems, circa-tidal rhythms are known to respond to environmental stimuli correlated with on-shore tides. Enright (1965) demonstrated that cycles of water turbulence, which simulate waves washing over the beach, can effectively entrain the circa-tidal activity rhythm of the isopod *Excirolana*. Other experiments indicated that cycles or changes of hydrostatic pressure cause behavioral responses in the amphipods *Synchelidium* (Enright, 1962) and *Corophium* (Morgan, 1965), and the pychnogonid *Nymphon* (Morgan *et al.*, 1964). However, these experiments did not show that such stimuli can entrain endogenous rhythmicity of the animals. Environmental variables coinciding with tidal cycles such as temperature might also affect synchronization processes.

The larval release activity of the terrestrial crab *Sesarma haematocheir* coincides with the times of high water at night, showing a unimodal tidal rhythm. A phase jump is involved in the timing process around the first and last quarters of the moon, so that the pattern of the tidal rhythm appears at 15-day intervals (Saigusa, 1982, 1985). When ovigerous females from the Seto population (Okayama Prefecture) are introduced by continuously dark conditions in the laboratory, they show a free-running rhythm whose phase at least initially is synchronized with times of nocturnal high water on the shore. The fact that the phase jump to the conjugated high water is reproducible under constant conditions suggests that its timing is also controlled endogenously. Artificial 24-h light-dark cycles cause a phase-shift of the circa-tidal rhythm. A striking aspect of the activity record is that the degree of the observed phase-shift closely corresponds to the time difference between lights-off and sunset. This indicates that 'dusk' is the critical signal for phase-setting in the circa-tidal timing systems of *Sesarma* (Saigusa, 1986).

A 24-h light-dark cycle thus functions as a zeitgeber (environmental cue), of *Sesarma* circa-tidal rhythm. However, there should be environmental cues other than

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the day-night cycle so that the daily timing of larval release may be synchronized with nocturnal high water. Two possible synchronizing agents were considered (Saigusa, 1985): (1) periodic changes in vibration or sound of surf washing on the beach, although Enright (1972) denied such a possibility; and (2) moonlight cycle, which is the zeitgeber for a semilunar rhythm. Previous experiments (Saigusa, 1986) were unable to demonstrate definite synchronization of *Sesarma*'s rhythm by artificial tidal cycles. Thus the next step was to determine whether this rhythm can be entrained by artificial moonlight.

This study attempted to establish the manner in which the endogenous tidal rhythm of *Sesarma* is synchronized by environmental cycles. For the 'control' experiment, populations of crabs were kept from spring in a 24-h light-dark cycle in the laboratory. Females that incubate eggs under these conditions are expected to lose synchrony with each other and show no clear tidal component in the larval release activity. In another experiment, crabs were maintained for several months under artificial moonlight cycles. If moonlight can synchronize *Sesarma*'s rhythm, the timing of release would be strongly coordinated to show a definite tidal component, and the requisite phase relations between the observed tidal rhythm and the artificial moonlight cycle would be the same as those between the tidal rhythm and the moonlight cycle in the field. Or if moonlight is an entraining agent of the tidal rhythm and the synchronization is possible up to the second or third release of larvae, then it may be possible to entrain a free-running rhythm of freshly collected populations. Furthermore, it may be possible to determine what component involved in moonlight cycles is actually significant for entrainment. Thus entrainment of a tidal rhythm of *Sesarma* by artificial moonlight cycles is the main focus of this article.

Saigusa (1980) showed that artificial moonlight cycle entrains a semilunar rhythm of larval release, *i.e.*, a remarkable semi-monthly variation in the number of females releasing larvae per night. However, this study was made using the Shima population, an inhabitant of the seacoast of the Pacific Ocean. It is unknown whether simulated moonlight cycles can similarly induce a semilunar rhythm in the Seto population which inhabits the seacoast of the Inland Sea. If artificial moonlight cycles evoke not only the tidal rhythm but the semilunar rhythm in both populations, then a question arises as to what relationships exist between the tidal and semilunar timing systems.

Field observations (Saigusa, 1982, 1985) demonstrated that the timing of larval release is synchronized with local tidal cycles. The habitat of the Seto population is situated around the central part of the Inland Sea, so that tidal cycles are delayed 4.5–5 h from those at Shima. The question also arises as to how moonlight cycles synchronize *Sesarma*'s rhythm and local tidal cycles.

Materials and Methods

Adult male and female crabs (*Sesarma haematocheir*) were collected from the field at Kasaoka, Okayama Prefecture, and Gokasho, Mie Prefecture. Collection sites are described elsewhere (Saigusa, 1980, 1982). In autumn crabs migrate to the hill near the riverside where larval release is performed, and hibernate in burrows dug on the ground or in narrow spaces among rocks or heaps of stones until the end of April. Crabs were dug out of the ground until April and captured in the thickets thereafter. Crabs were then transferred to a light and temperature controlled laboratory (luminous intensity at about 700 or 1200 lux on the floor in the light period of 24-h LD cycles). Crabs were placed in an aquarium equipped with a shallow pool on one side (to facilitate ecdysis) and hiding places made of boards on the other. Crabs were fed daily. Temperature was maintained at 23 ± 1.5°C for most experiments.

Once copulation is finished, ovulation occurs and fertilized eggs are attached to ovigerous hairs in the folded abdomen; this is termed 'onset of incubation' or 'egg-production.' The female then carries a clutch while eggs undergo embryonic development. Each female was examined daily for the onset of incubation, and ovigerous females were separated in small plastic containers (70 cm long, 40 cm wide, and 25 cm high) containing a small amount of very diluted seawater (see Fig. 1 in Saigusa, 1980). The color of the eggs was examined every few days. Females with lustrous, brownish green eggs (signaling the onset of larval hatching) were transferred to another container. Those animals whose eggs were estimated to hatch within a few days were individually placed in the recording apparatus to monitor the time of larval release.

The larval release recording system consisted of a sensor unit (infrared source-receiver) placed within the experimental chamber, and a photoelectric-switch-amplifier unit placed outside the chamber. The latter unit detects and responds to a decrease in the transmitted light beam following the release of zoea-larvae. In the present experiments OPE-S100 or E3S models (Omron Co. Ltd., Japan) were used as the sensor unit. The output of the photoelectric switches was monitored by an event recorder (Saigusa, 1986). Crabs were confined within the chamber throughout the experiment.

An incubating crab was placed in the recording apparatus until larval release occurred. The glass beaker was replaced and a new ovigerous female was transferred from the container to the recording apparatus after larval release. This procedure was performed during the light

period of 24-h LD cycles. The females who had completed larval release were placed in another container where they incubated the next clutch without males. Subsequent procedures were the same as those for the first incubation. Experiments were performed using four chambers fitted with 6–15 recording instruments.

To test whether the daily timing of larval release by females can be affected by moonlight, the chamber was illuminated with dim light produced by a midget lamp—the same used for entrainment of a semilunar rhythm in the Shima population (Saigusa, 1980). The intensity of this artificial moonlight remained constant at 0.1–0.15 lux at the floor and thus did not mimic the phases of the moon.

The trend of activity pattern produced by the treatment of the artificial moonlight was estimated by calculating least-squares regression lines through sequential activity records. The slope of each regression line ($y = ax + b$) is given by the following equation:

$$a = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\Sigma(x_i - \bar{x})^2}$$

where: x_i and y_i indicate a coordinates used to express each point of the larval release activity (the origin is taken on an intersection of the date-axis and the time-axis); $\bar{x}$ and $\bar{y}$ show mean values of x_i and y_i, respectively. The deviation of each point of the activity from the regression line was estimated along the abscissa where the point is plotted, and the variance (V) of those estimates was calculated for every regression line. In this paper, this value (a unit: h) is used as an index of the degree of synchronization among individuals.

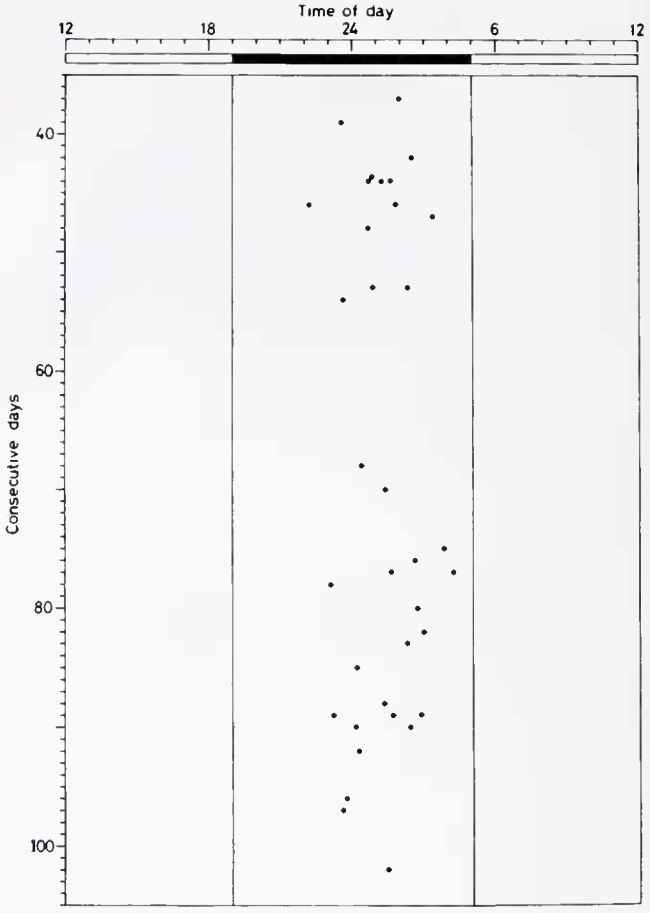


Figure 1. Daily timing of larval release by the Seto population monitored under the condition of a 24-h LD alone. A black dot indicates the time of day of larval release by a female. Vertical axis: number of days after the population was introduced to the laboratory.

Results

For the 'control' experiment, male and female crabs were collected at Kasaoka on 6 May 1984 and confined in the experimental chamber. They were kept under an artificial light regime (LD 14:10) which does not include moonlight. Under this condition the first incubation occurred from May to July. Each female incubated eggs for one month and then released them as zoea-larvae. Most females subsequently carried a second clutch and released larvae after the same period. Larval release occurred at night (Fig. 1)—especially in the latter half of the night. The release was not synchronized and the overall activity pattern gave no clear indication of a tidal component.

To evaluate the effect of artificial moonlight cycles on the timing of larval release activity, crabs (60 males; 100 females) were collected at Kasaoka on 24 April 1983 and transferred to the laboratory. They were kept under a light regime which included a 24.8-h artificial moonlight cycle at night. The initial photoperiod applied to the animals was LD 12:12. Under this condition 30 females started their first incubation from the 22nd to the 37th day and released larvae between the 53rd and the 66th day (see Fig. 5B). As indicated in Figure 2A, the simulated moonlight cycle brought marked changes in the daily timing of the activity: a negative slope is clearly seen from the nocturnal activity pattern.

In this experiment no incubation occurred after the 38th day (1 June). Since it was evident that the short-day condition inhibited egg-production, the day-length was changed to LD 15:9 from the 67th day (30 June). This treatment induced ovulation after two weeks. Such an inhibitory action by the photoperiod also occurred to the animals shown in Figure 2A: these females did not incubate the second clutch immediately after the release of larvae, but required at least one month of exposure to the long-day conditions.

The artificial moonlight program adopted in the short-day conditions (Fig. 2A) was composed of an idealized period of 24.8 h. For long-day conditions, however, the

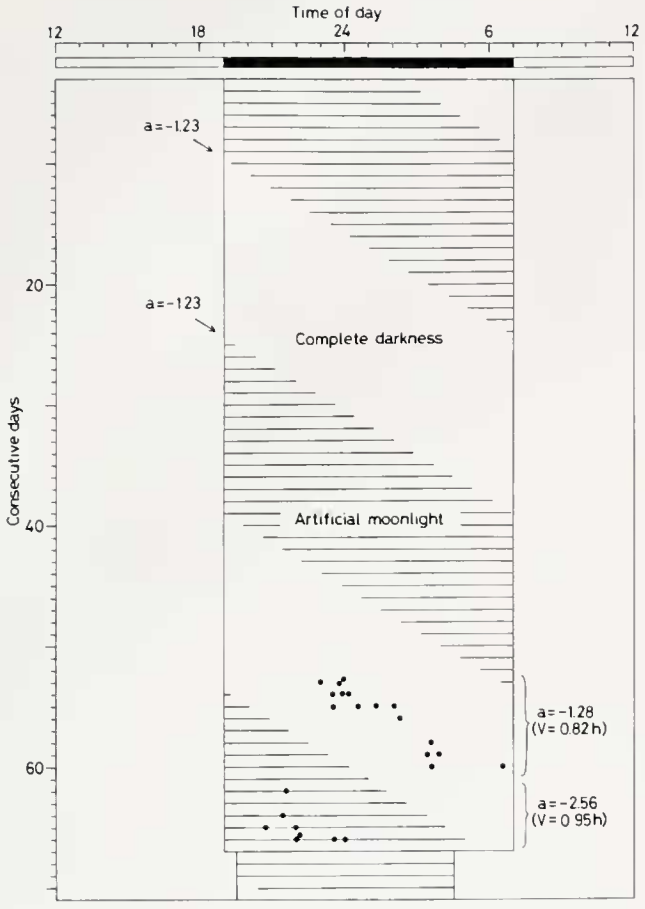


Figure 2(A). The larval release activity by the Seto population subjected to an artificial moonlight cycle of 24.8 h. Horizontal bars show the times when the experimental chamber was illuminated by the artificial moonlight. 'On' and 'off' times of this moonlight were manually set every day using a timer. *a*: The slope of retardation of 'on' or 'off' of the artificial moonlight (left side), and the slope of each tidal component (right side). *V*: Variance of the spawning activity along the regression line.

period of the simulated moonlight cycle had to be modified so that it could recur at 15-day intervals. In this experiment, such a modification was based on data from the Annual Table on Scientific Affairs (Japanese name: Rika Nenpyo, ed. by Tokyo Astronomical Observatory): times of 'rise' and 'fall' of the moon were replaced by the times of 'on' and 'off' of the artificial moonlight, respectively. This is the same kind of zeitgeber program as used previously for entrainment of a semilunar rhythm (Saigusa, 1980). Of the data described in this table, those from June–September were continued from the initial program.

Figure 2B summarizes the daily timing of larval release activity recorded under the new light regime which included a 24.5-h artificial moonlight at night. At first glance the activity corresponds to the phase of the moonlight cycle, showing a pattern with a negative incline at semi-monthly intervals. In this population, the slope of each sequential activity pattern was close to that of the artificial moonlight cycles. Thus the fact that the activity of a group of crabs was transformed from apparently arrhythmic (Fig. 1) to clearly coordinated (Figs. 2A, B) demonstrates that the moonlight cycle is adequate to be the zeitgeber of the circa-tidal rhythm of *Sesarma*.

Field studies (Saigusa, 1982) established that the tidal rhythm of the Seto population causes a phase jump of 7–

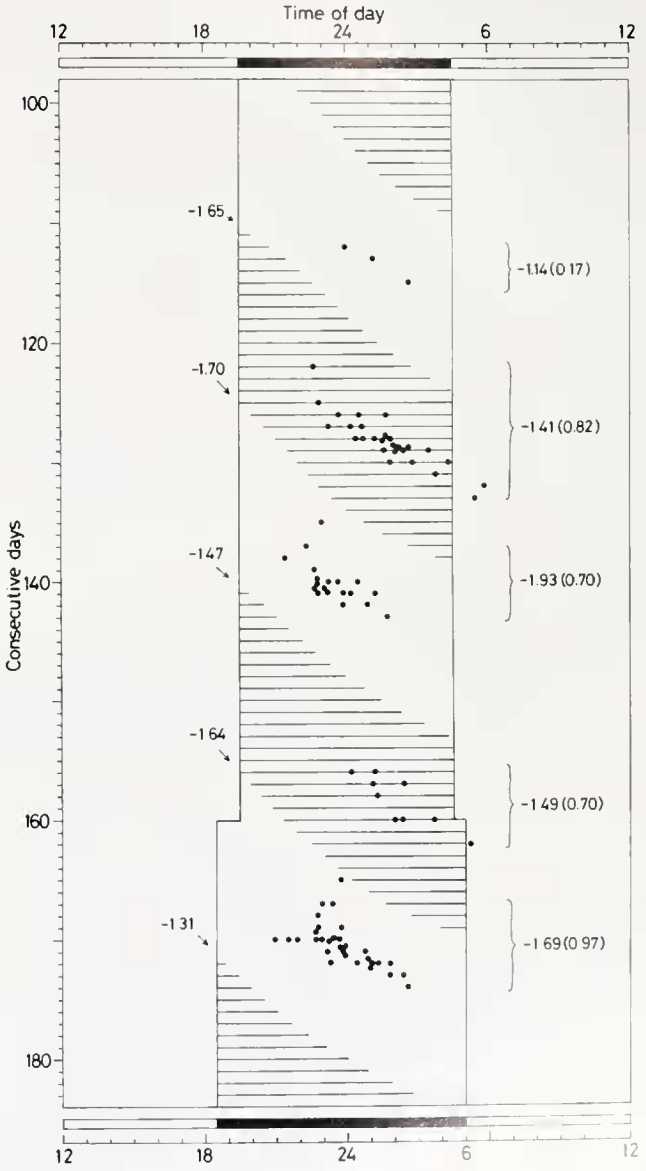


Figure 2(B). Daily timing of larval release by the Seto females recorded under an artificial moonlight cycle of about 24.5 h. Vertical axis: number of days after the population was brought from the field (the data continued from Fig. 2A). Slope of each tidal component and variance of the activity are shown on the right side of the corresponding data (the data on days 135 and 165 are not included in calculation of regression line). Temperature was constant in the region of 21 ± 1.5°C until day 65, and about 23 ± 1.5°C thereafter.

8 h around the first and last quarters of the moon. The activity appears immediately after dusk for 3–4 days before the afternoon high tide advances to the night. The long-duration records in the laboratory, however, showed no clear indication of the timing synchronized with the onset of darkness. The records of days 165–168 illustrated in Figure 2B, for instance, show that the activity occurred more than 4 h after lights-off. Furthermore, as seen in the records of days 133–134 and day 162, the activity occurred beyond the dark period. These phenomena, which have not been observed in the field, happened often during the experiments using the Seto population.

Experiments to examine the effect of artificial moonlight cycles on the daily timing of the activity were also conducted using the Shima population. For this purpose male and female crabs were captured on 7–8 May 1986 from the field and transferred to the laboratory. They were divided into two groups: one was kept under the artificial light regime without moonlight (LD 15:9), and another was exposed to a 24.5-h artificial moonlight at night (LD 15:9). The group kept for a long time under a 24-h LD condition alone is expected to show no clear tidal component in the activity pattern. On the other hand, if moonlight is an essential stimulus synchronizing *Sesarma*'s rhythm, the group exposed to artificial moonlight would exhibit a distinct tidal component at night.

Without moonlight, Shima females released larvae at night; most release occurred during the first half of the night (Fig. 3). The activity pattern did not show a detectable tidal component throughout the period. However, another group from the Shima population synchronized its release to the artificial moonlight cycle (Fig. 4), yielding a distinct tidal component at semi-monthly periods. A striking feature on comparing the data illustrated in Figure 4 with those of Figures 2A and B is that the evoked tidal rhythms in the two populations had different phase relations to the artificial moonlight cycles; the phase difference was about 4–5 h. The fact that the slopes of the tidal component in the Shima population were larger than those of the Seto population would mean that the period of the Shima population tidal rhythm is closer to that of a so-called daily rhythm.

Figure 5A summarizes the variation of the number of Shima females releasing larvae per night. The 'control' experiment (upper panel) group did not show a clear rhythmicity either in the onset of incubation or in the timing of larval release. On the contrary, the group treated with artificial moonlight (lower panel) exhibited a well-defined semi-monthly variation in the larval release activity, and the fluctuation corresponded to the varying phase relations between the 24-h LD and artificial moonlight cycles. For this group the number of females that started incubation also fluctuated at semi-

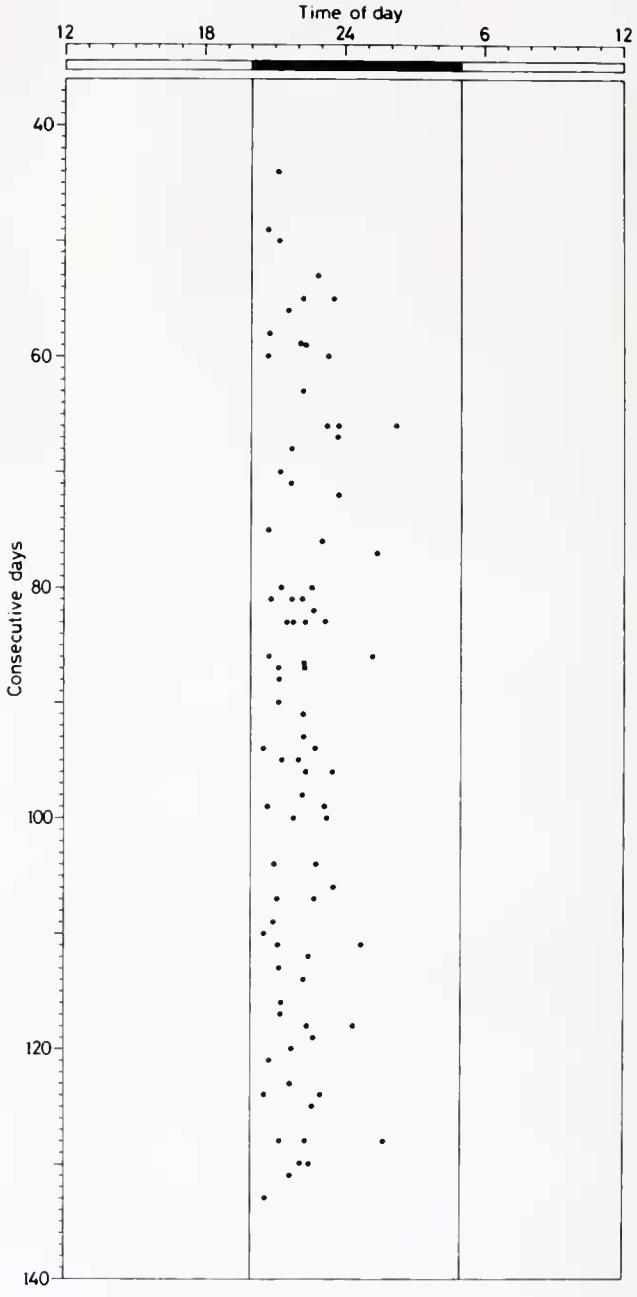


Figure 3. The larval release activity of the Shima population monitored under the condition of a 24-h LD cycle containing no moonlight. The data show two spawnings by most females and a third spawning by a portion of them.

monthly intervals. The semilunar rhythm of ovulation is certainly weaker than that of the larval release. Moreover, when the onset of incubation was delayed (an arrow indicated in Fig. 5A), some females were included in the group animals that constituted the next peak of the larval release. Nevertheless, the trend in Figure 5A suggests that the semilunar rhythm of larval release is ba-

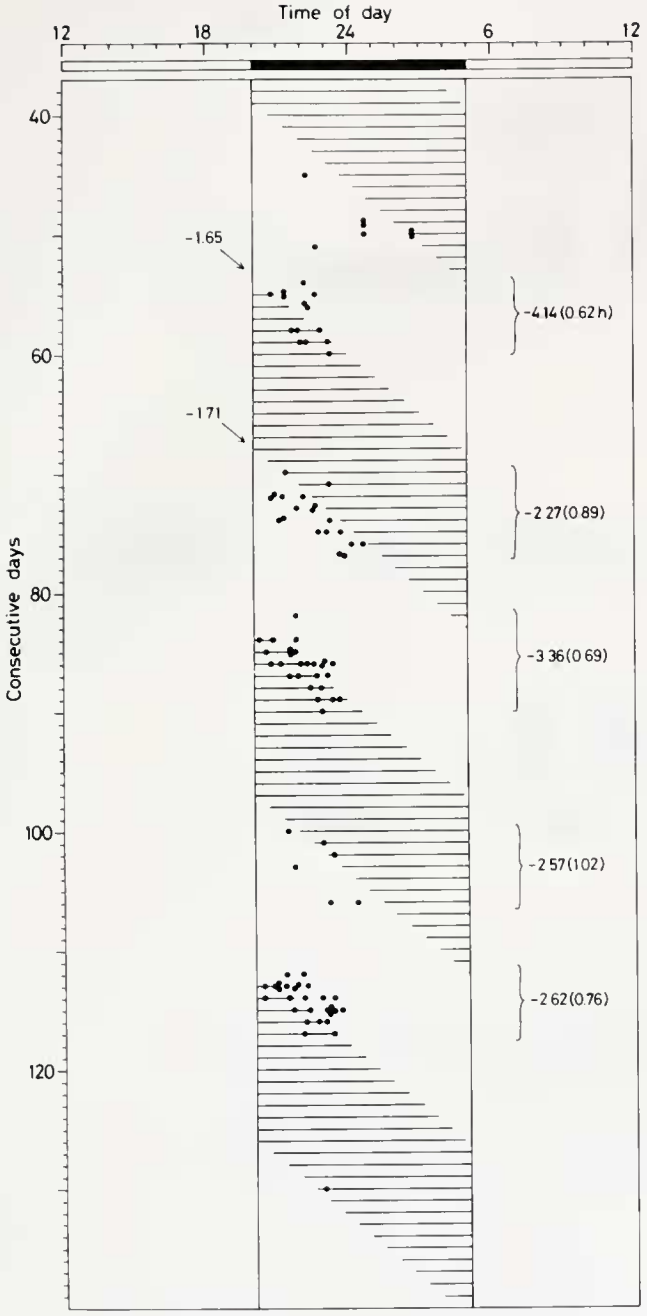


Figure 4. The larval release activity of the Shima population subjected to an artificial moonlight cycle of about 24.5 h. Horizontal bars indicate the artificial moonlight. Slope of the retardation of this moonlight is shown by arrows on the left side of the data. Slope and variance in each tidal component are indicated on the right side of the corresponding data. Vertical axis: number of days after 8 May.

sically due to the occurrence of the semilunar rhythm of ovulation.

A similar fluctuation was also obtained from the data of the Seto population treated with artificial moonlight (Fig. 5B). In this experiment a short-day condition inhibited egg-production so that no female released larvae between the 68th and the 111th day. In a long-day condition three distinct peaks (A, B, and C) and a small peak (D) appeared in the larval release activity. Peaks A and B consist of the spawning by the females which first began incubation during the long-day condition and the second spawning by females that hatched out the first brood during the short-day condition, respectively. Most females in group A were added to group B at the onset of the second or third incubation, so that the peak D became vague. Each peak of the larval release activity in this population is not so clearly separated as that of the Shima population semilunar rhythm (Fig. 5A). This suggests that the semilunar timing of the Seto population is somewhat weak compared with that of the Shima population.

Previous experiments (Saigusa, 1986) demonstrated that the timing of larval release in *Sesarma* is controlled endogenously, thus the synchronized release in Figures 2A, B, and 4 is not a direct response of the animals to the moonlight stimuli. But the question remains: what component of the moonlight cycles synchronizes *Sesarma*'s tidal rhythm? Since the moonlight cycles in Figures 2 and 4 are unnatural due to a lack of cyclic variation in light intensity, it is possible that the tidal rhythm is synchronized by (1) moonlight illumination for some days around the full moon (*ca.* 30-day period), or (2) by retardation of the times of rise and/or fall of the moon (*ca.* 24.5-h to 24.8-h period).

To answer this question, the animals were exposed to two kinds of simulated moonlight cycles. At first, effects of the moonlight at the time of the full moon were examined. Crabs were collected at Kasaoka on 24 April 1986 and exposed to artificial moonlight throughout the night (9 h) for one week every 30 days. If the retardation of the times of rise and fall of the moon is important for entrainment, then the larval release activity should show no definite tidal component under such a condition. However, the results (Fig. 6A) indicated a tidal component at semi-monthly intervals. As seen from values of the variance, individual animals are not so strongly synchronized as those monitored under 24.5-h moonlight cycle (Fig. 2B). This indicates that such a moonlight cycle (Fig. 6A) weakly entrains *Sesarma*'s rhythm.

In the next two experiments the same kind of zeitgeber program was adopted for both Shima and Seto populations collected on 11–12 May and 14 May 1987, respectively. As indicated in Figures 6B and C, each chamber was illuminated by dim light through the night (9 h) for four days each month. Unlike the results shown in Figure 6A, the tidal component was not detectable from either of these experiments. In another experiment the Shima population (collected 11–12 May 1987) was exposed to a moonlight cycle simulating the time of the new moon. This experiment also failed to demonstrate a well-de-

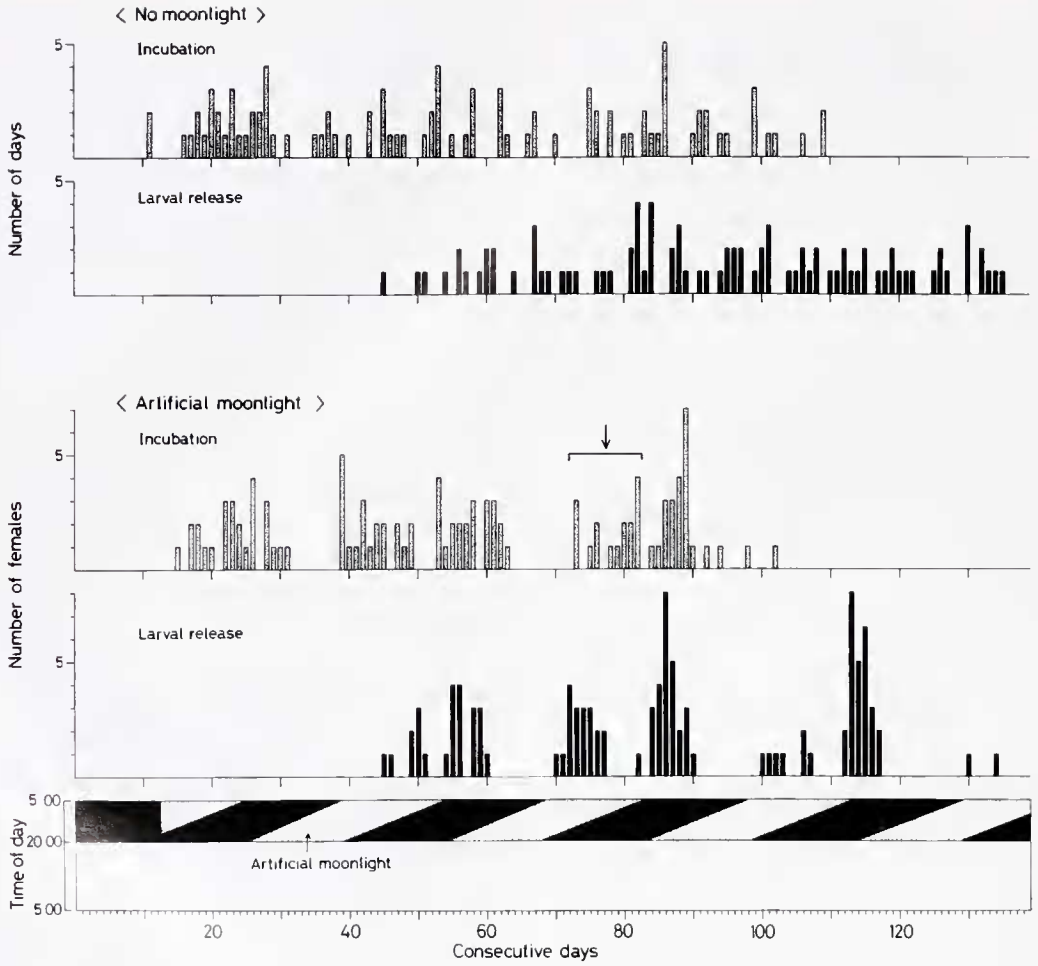


Figure 5(A). Plots of the number of females starting incubation per day and the number of females releasing larvae per night (Shima population). Upper panel: experiments without moonlight. Lower panel: experiments with a 24.5-h artificial moonlight cycle. Moonlight program is shown at the bottom of the figure. The data of larval release are based on those of Figures 3 and 4, respectively. The number of females does not precisely agree with the number of black circles in corresponding dates of those figures, because there were some females whose larval release was not monitored. All females were marked in these experiments. Periods of incubation in each female (upper panel) were 30.4 days for the first brood, 27.8 days for the second brood, and 26.8 days for the third brood, respectively, in the mean. Incubating periods were similar in the group exposed to moonlight (lower panel): 30.9 days, 28.7 days, and 27.0 days, respectively.

fined tidal component (Fig. 6D). These data probably suggest that moonlight cycles that simulate either the full moon or the new moon have a lesser effect on entrainment than the moonlight cycle shown in Figures 2 and 4.

The reason a tidal component is seen in one case (Fig. 6A) and not the other (Fig. 6B) for same population is because of the difference in the time of collection. While the former experiment (Fig. 6A) began on 24 April, the latter (Fig. 6B) commenced on 14 May. (In both experiments artificial moonlight was administered around the time of the half moon. In the former experiment the first moonlight cycle was supplied before the full moon in May, *i.e.*, around the first quarter; in the latter experiment it was given around the last quarter in May.) Crabs would have been more strongly synchronized with natural moonlight cycles during the three weeks. This would have delayed entrainment in the latter experiment, which made it difficult to evoke a tidal rhythm during the three times of spawning.

Figure 7 summarizes the fluctuations of the number of females that released larvae under these zeitgeber programs. A weak semilunar rhythm is distinguishable from the data of the Seto population collected in April (Fig. 7A), but no clear rhythmicity appears in the other data (Figs. 7B, C, D). The fact that the semilunar rhythm appears at the time the tidal rhythm is entrained suggests that these rhythms are not separately but simultaneously entrained by the moonlight cycle.

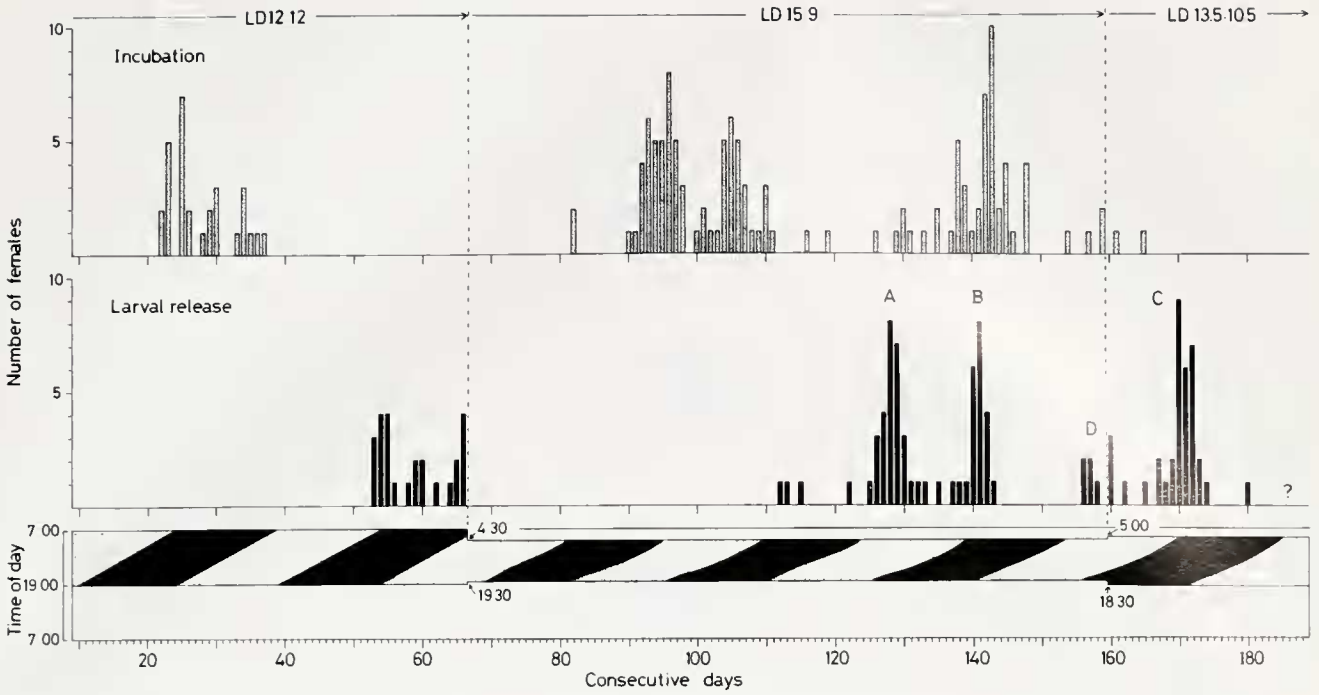


Figure 5(B). Plots of the number of females incubating a clutch and releasing larvae under simulated moonlight cycles (Seto population). Moonlight program applied to the animals is shown at the bottom of the figure (artificial moonlight is indicated by dotted area). The data of larval release were based on Figures 2A and B. Time of larval release was not monitored in a portion of females, so that the number of females releasing larvae per night does not precisely agree with the data shown in those figures. See text for further details.

All experiments reported above could not determine the process until individuals were synchronized by exposure to the artificial moonlight. One approach to this question is to apply the 24.5-h moonlight cycle to a freshly collected population showing a free-running tidal rhythm. For this purpose, ovigerous females (66) were collected at Kasaoka on 8 July 1986 and exposed to the artificial moonlight. In this experiment, the lights-off time in the 24-h LD cycle was set at 20:00. As the 'control' experiment, ovigerous females were collected at the same site on 12 August 1987 and maintained under the 24-h LD cycle alone (Fig. 8, right panel). If the animals were not exposed to artificial moonlight in this condition, *i.e.*, lights-off at 20:00, the larval release rhythm would free-run approximately in phase for 1–2 h delayed from the times of high water in the field (see Saigusa, 1986). On the other hand, entrainment to the artificial moonlight would be recognized when the phase relationship between the tidal rhythm and the moonlight cycle resembles those in Figures 2A and 2B. As Figure 8 (left panel) indicates, it took more than one month for the tidal rhythm to reach such a state after artificial moonlight was administered. Slopes of the new phase and variance of each activity were also similar to those recorded in Figure 2. A comparison of the data summarized in both panels (Fig. 8) represents a slight but clear discrepancy between the new phase and free-running phase in relation to the times of high tide. Therefore, it may be concluded that the left panel of Figure 8 shows a process where the tidal rhythm is phase-shifted and then entrained by the artificial moonlight. This process requires a long period even under the 24.5-h moonlight cycles.

Discussion

Data like those in Figures 2, 4, and 8 suggest that the females may have been responding to the retardation of the moonlight by 0.5 h to 0.8 h nightly. Previous data on entrainment of a semilunar rhythm (Saigusa, 1980) were similarly interpreted. However, retardation was not necessarily required to entrain the tidal and semilunar rhythms in Figures 6A and 7A. Ecological considerations may also favor the view that environmental stimuli such as moonrise and moonset are unsuitable synchronizing agents for the larval release rhythm of *Sesarma*. The intensity of moonlight would be extremely weak or nonexistent at the times of rise and fall of the moon even during the days of full moon. Furthermore, it is common that low clouds irregularly hide the moon at night. In such cases, it may be impossible for the animals to distin-

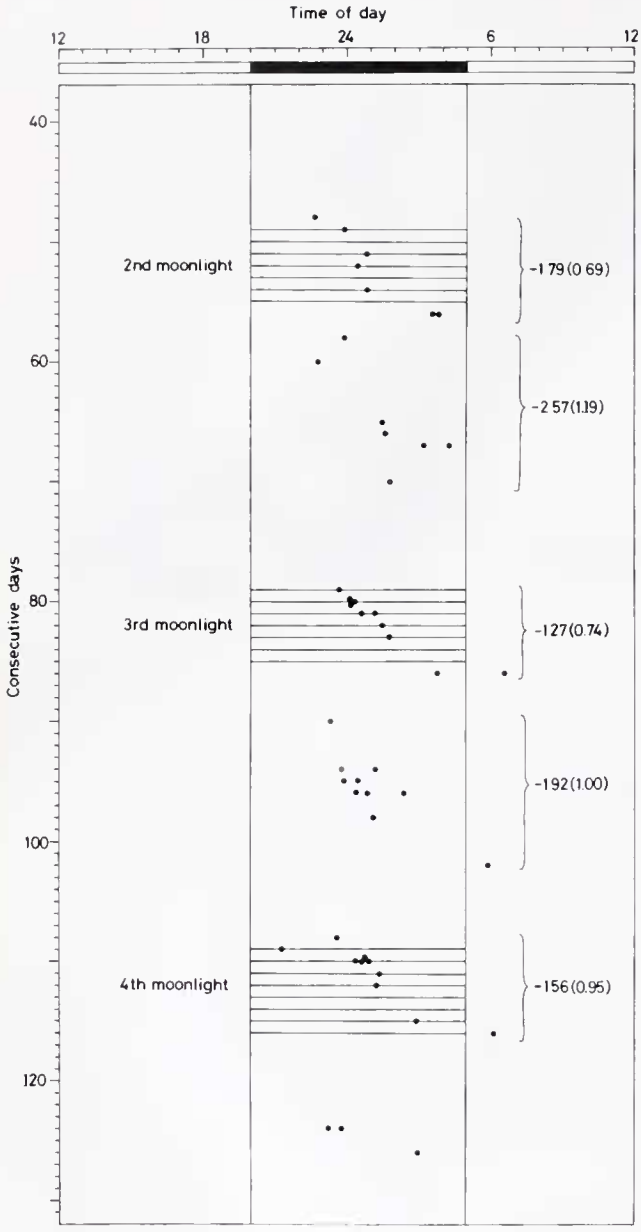
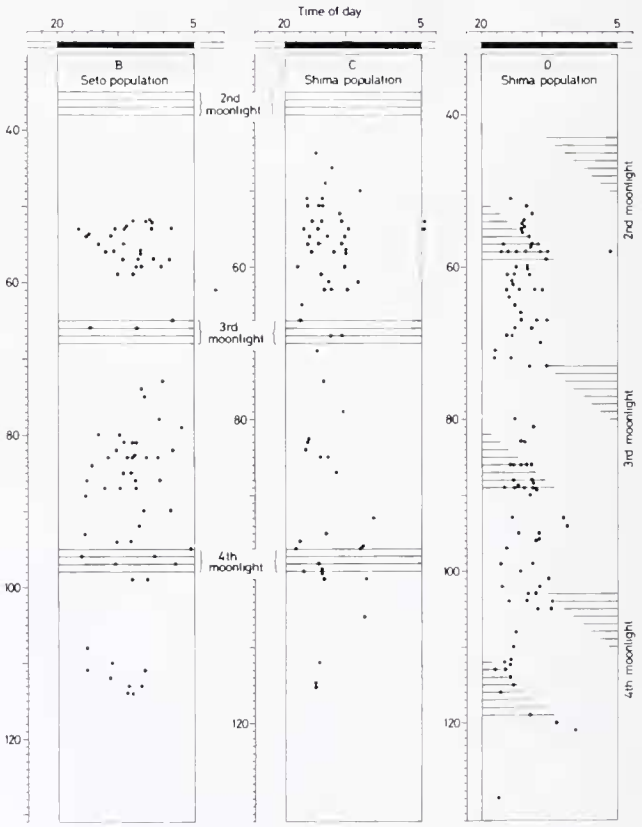


Figure 6(A). Time of day of larval release by the Seto population monitored under an artificial moonlight cycle. Moonlight is shown by the horizontal bars in the dark period of the 24-h LD cycle. Slope and variance of the activity pattern are shown on the right side of the corresponding data.

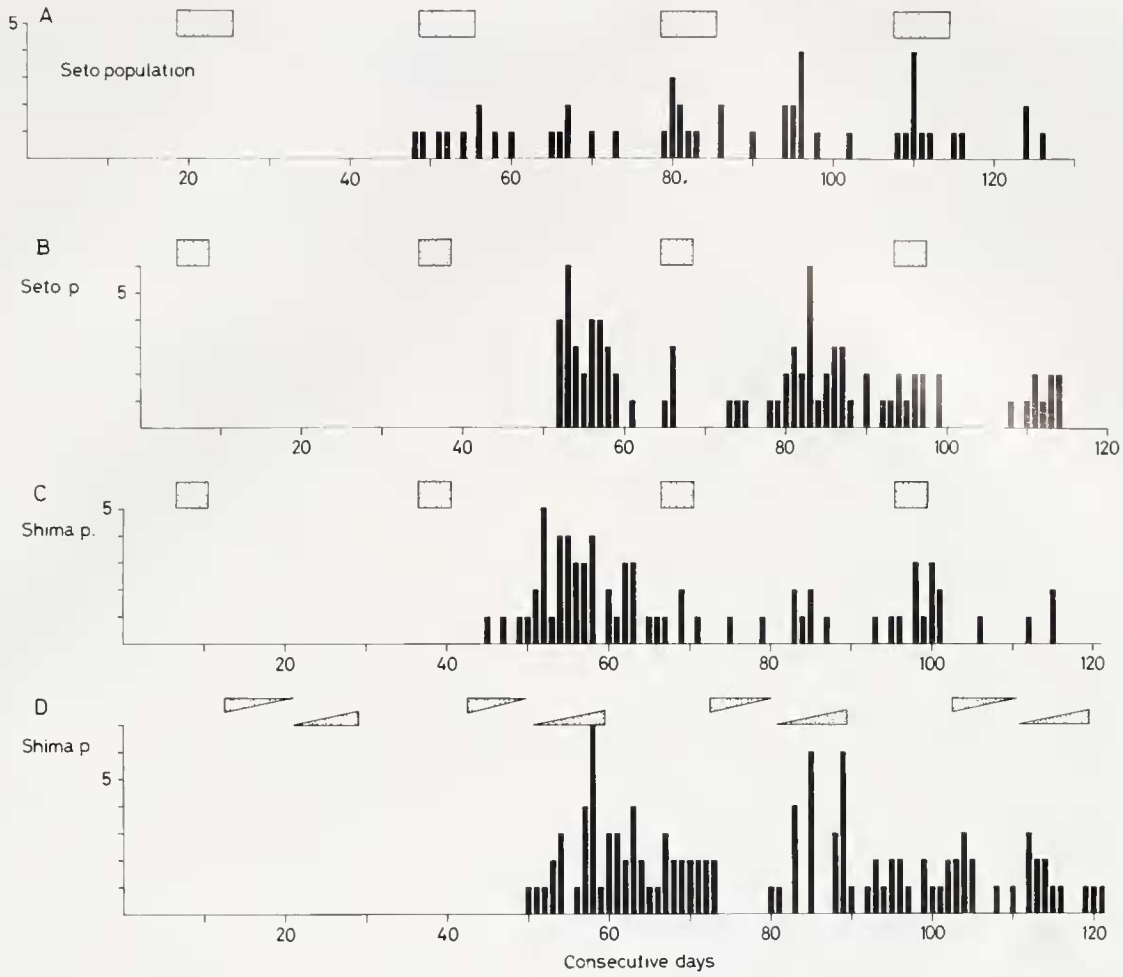
guish which is the real moonrise or moonset. This study certainly demonstrates that even a moonlight illumination for some days around the time of full moon functions for entrainment. But it could not determine the essential component for the entraining agent involved in moonlight cycles.

The pattern of the larval release rhythm of the Shima population (Figs. 4, 5A) differs from that of the Seto population (Figs. 2, 5B) as follows: (1) tidal component appears in the first half of the night; (2) the population has somewhat strong semilunar timing. Since the same zeitgeber cycles were given to both populations, such a difference in the pattern of the rhythm may be based on the properties specific to each population. The larval release pattern of the Shima population is clearly similar to that of the Izu population (Saigusa, 1985), which suggests that the pattern illustrated in Figure 4 is common to populations inhabiting the coast of the Pacific Ocean.

Another striking aspect on comparing the pattern of the tidal rhythm between Seto and Shima populations is different phase relations to the artificial moonlight cycles. Figure 9 represents times of high tide occurring at the seacoast near the habitats of each population. Kasaoka is located around the central part of the Seto Inland Sea, so that while the day-night and moonlight cycles differ by only 10 min between both areas, times of high tide are 4.5–5 h behind those at Shima. The degree of the phase difference between the evoked tidal rhythms in both populations clearly correspond to the time lag of the tidal cycles between these locations. Based on population-specific reactions to the moonlight cycle, this re-



Figures 6(B–D). Daily timing of larval release by Seto and Shima populations recorded under artificial moonlight cycles. Artificial moonlight is indicated by horizontal bars. Vertical axis: number of days after the animals were introduced to the laboratory.



Figures 7(A–D). Plots of the number of females releasing larvae per night (Seto and Shima populations). Artificial moonlight applied to the animals is indicated by the stippling on top of each data. The data are based on those of Figures 6A–D, respectively.

sult would give a sufficient explanation for synchronization of the daily timing of larval release with local tidal cycles.

Experiments on entrainment by simulated moonlight cycles, however, lead to a strong suspicion that local tidal cycles also can affect the timing of larval release. As field studies indicate, the pattern of *Sesarma* tidal rhythm is precisely synchronized with the times of high tide at night, especially for the Seto population (Saigusa, 1982). Hence, it seems that each female, at least for the Seto population, is capable of perceiving some information for precise timing from the tidal cycles near its habitats. Otherwise it would be impossible for this population to keep a precise phase relationship between the larval release rhythm and local tidal cycles over many generations. Nevertheless, possible entraining agents originating from tidal cycles are limited; the only remaining possibility was a periodic change in sound or vibration of surf which is carried to land (Saigusa, 1985). The activity pattern obtained under the artificial tidal cycles mimicking such stimuli, however, were less easily reconciled with tidal cycles as an entraining agent for endogenous timing (Saigusa, 1986). This might imply that such stimuli are too simple to function as a zeitgeber, otherwise they have no effect at least on internal timing.

Timing mechanisms underlying the circa-tidal rhythm of *Sesarma* have been described in terms of circadian systems (Saigusa, 1986). This model assumes two 'circadian' oscillators having a slightly different period to each other. The α-oscillation (*ca* 24-h period), a so-called driving element, is subject to the environmental 24-h LD cycles. On the other hand, the β-oscillation, a driven element, is coupled to, and phased by, the α-oscillator. Larval release is linked to a phase of the β-oscillator. The period of this oscillator is about 24.5 h. (In my 1986 paper, it was described at about 24.8 h. However, because

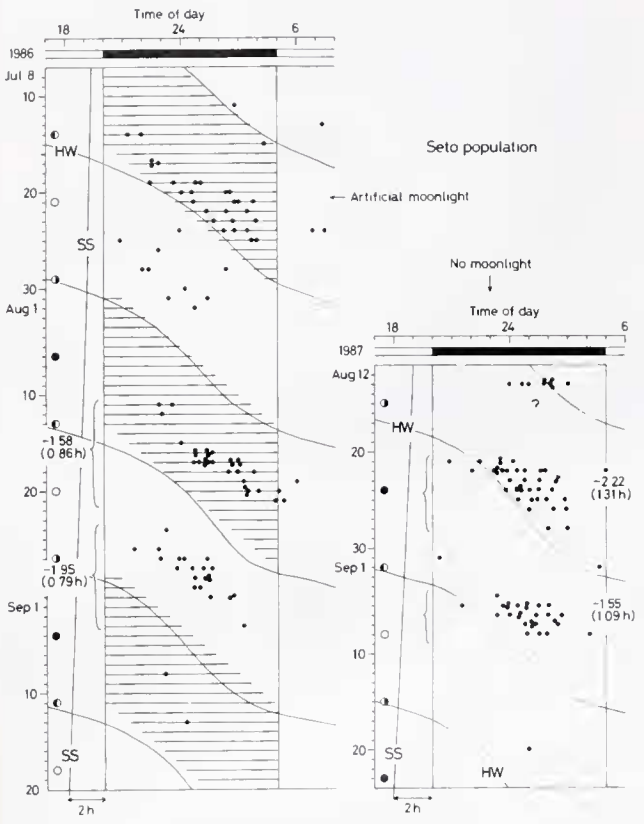


Figure 8. Time of day of larval release by the Seto population monitored under the 24.5-h moonlight cycle (left panel) and without moonlight (right panel). Diagonal lines (HW) connect the times of high water which should occur in the field at Kasaoka. *SS* connects the times of sunset in the field. Horizontal bars (left panel): artificial moonlight. Slope and variance in the activity pattern are presented on the left or right side of the corresponding data. In 1986 experiments these values were not calculated at the first half of the experimental period where no clear tidal component is seen an the activity pattern. Open circle, darkened circle, and semi-circles represent full moon, new moon, and the first or last quarters of the moon, respectively.

this oscillation follows the nocturnal high tide up to 7–8 h over the course of 15 days, it would be better to consider its period to be about 24.5 h in the Seto population. In the Shima population, slopes of the tidal rhythm were larger than those of the Seto population. This suggests that the period of β-oscillation is closer to that of the daily rhythm.) This hypothesis may be, indeed, an extention of the coupled circadian oscillator model which was developed for the daily rhythm of *Drosophila* eclosion (Pittendrigh and Bruce, 1959; Pittendrigh, 1960). But the important factor is the assumption that the driving oscillator postulated in *Sesarma* circa-tidal systems leads to the other oscillator which clearly differs in period, though slightly. This raises the critical question of what justification there is for such an assumption which has not been considered previously in circadian rhythms. In addition, the concept of 'oscillator' is not so specified as in Pittendrigh's model. For these reasons, *Sesarma*'s rhythm has been formulated in terms of α and β oscillators, not A and B oscillators.

According to this model, the data from Figures 1 and 3 regarding experiments in which the animals were kept for a long time under a 24-h LD cycle alone, is accounted for as a state in which the driven β-oscillator loses synchrony among individuals, though the α-oscillator is ap-

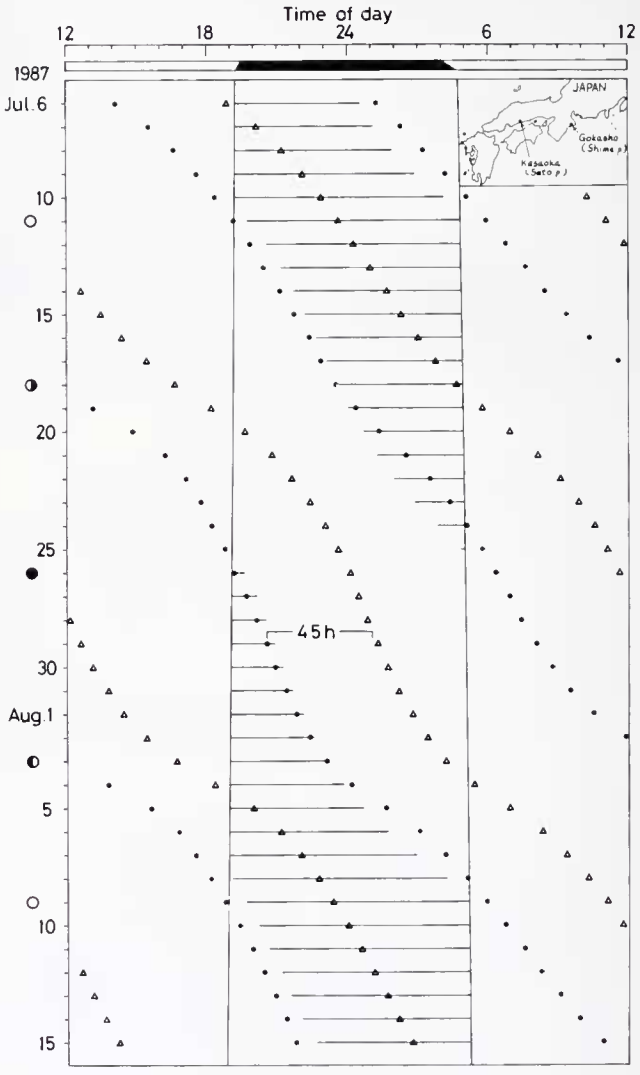


Figure 9. Plots of the times of high tide at the seacoasts of Gokasho (lat. 34°19″ N, log. 136°40″ E) and Kasaoka (lat. 34°30″ N, 133°30″ E). Day-night cycles (vertical lines) and natural moonlight cycles (horizontal bars) are drawn based on Gokasho data (the moonlight in the daytime is omitted). These environmental cycles are delayed only 10 min at Kasaoka. To avoid confusion, the data of Kasaoka are not illustrated. Dark circles and open triangles show the times of high tide occurring at Gokasho and Kasaoka, respectively. Phase relations between tidal and moonlight cycles in a certain location is about constant throughout a year; in this figure the data of July–August are summarized. Open circle, darkened circle, and semi-circle on the left side represent full moon, new moon, and half moon, respectively.

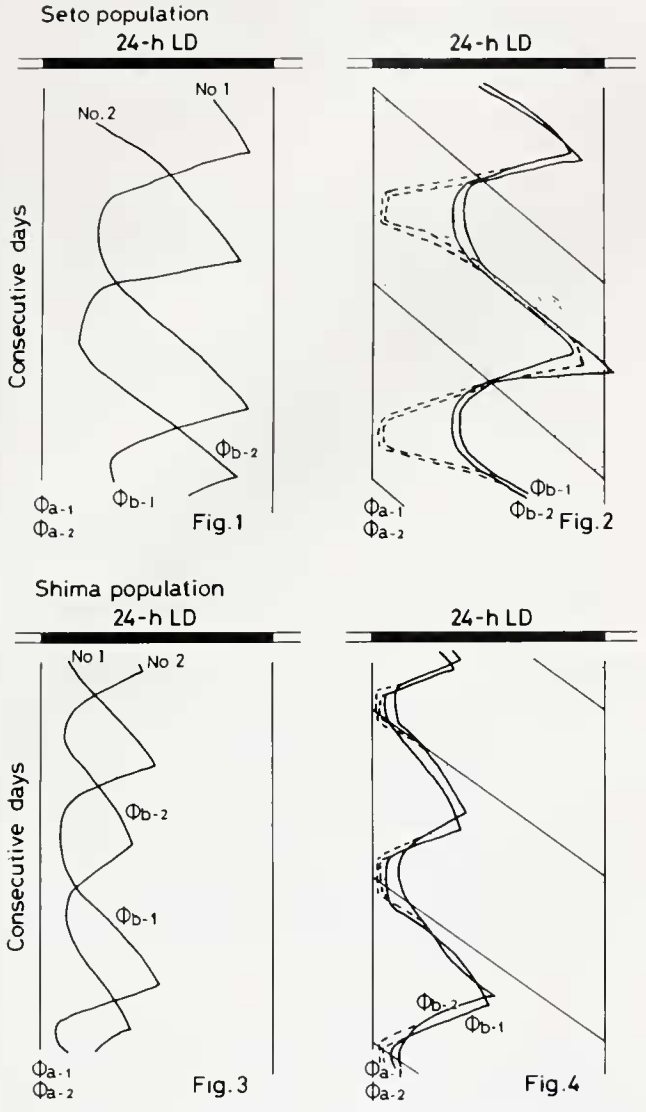


Figure 10. Explanation of *Sesarma* tidal rhythm in terms of coupled circadian oscillatory systems. Left panel: interpretation of the data shown in Figures 1 and 3 with examples of two individuals. Right panel: interpretation of the data illustrated in Figures 2 and 4 with examples of two animals. ϕ_{a-1} and ϕ_{a-2}: a phase point of the α-oscillator corresponding to the time of sunset in each animal. ϕ_{b-1} and ϕ_{b-2}: an arbitrary phase point of the β-oscillator which is assumed to determine the time of day of larval release in each female. The right panel shows a state where the β-oscillation is somewhat awkward upon the phase jump to the times of sunset in comparison to the natural conditions (the broken curves in the figure). Artificial moonlight is shown by dotted area.

parently synchronized with the light-dark cycle (Fig. 10, left panel). On the other hand, the data presented in Figures 2 and 4 provide obvious grounds for considering the phase of the β-oscillation to be coordinated by the moonlight cycle. A functional dichotomy between the driving and driven oscillators further suggests—in order to transform timing from being apparently arrhythmic to strongly coordinated—that the β-oscillator feeds back to the α-oscillator. In *Sesarma* circa-tidal rhythm, this process would require considerable time.

The tidal rhythm of *Sesarma* involves a phase jump around the first and last quarters of the moon (Saigusa, 1982, 1985). This phase jump was reproducible in constant conditions in the laboratory, which suggests that this timing is controlled endogenously (Saigusa, 1986). Field observations demonstrated that after the phase jump the timing of larval release is synchronized with dusk until the high water approaches night. As shown in Figure 2B, there was no clear indication of the activity coinciding with the light-off time in the 24-h LD cycle. In some cases the activity intruded into the light period. These laboratory observations indicate that the timing of the activity becomes somewhat awkward in its phase jump to the time of lights-off under the artificial conditions. If one accepts the reality of such a possibility, then the observed activity pattern would be explained in the manner shown in the right panel of Figure 10.

The main feature of the record illustrated in Figure 6A is that the tidal rhythm of *Sesarma* can be entrained by a moonlight cycle of one week occurring every 30 days. The question remains why a tidal component appeared in the activity pattern in the absence of either a 24.5-h or a 24.8-h component in the artificial moonlight cycles. The most obvious answer is that the circa-tidal rhythm of *Sesarma* itself involves an endogenous semi-monthly modulating component, which has been synchronized with the moonlight cycle. In other words, entrainment of a semilunar rhythm inevitably evokes a tidal rhythm in the timing systems of *Sesarma*.

Several workers (*e.g.*, Naylor, 1958; Bünning and Müller, 1961; Barnwell, 1968) have proposed that lunar and semilunar rhythms arise due to the occurrence of two separate rhythmic systems in an organism: one with a circadian period (12 h or 24 h), and another with a circa-tidal period (12.4 h or 24.8 h). Based on analogies of two physical oscillators, the superposition of these endogenous rhythms have been postulated to produce long-period rhythmicities as an additive effect in which the period differs slightly from each other. Field studies (Saigusa, 1982, 1985) certainly demonstrated that *Sesarma* tidal rhythm involves a daily component as well as a tidal component. However, on the basis of the response of this rhythm to 24-h LD cycles, there was no obvious ground for considering such an activity pattern to consist of an interaction of circadian and circa-tidal rhythms; a circadian rhythm with a period of 12.4 or 24.8 was sufficient to account for the animal's tidally timed activity (Saigusa, 1986). Hence, the hypothesis that the pattern of the semilunar rhythm of *Sesarma* (Figs. 5A, B, 7A) is due to superposition of daily and tidal rhythms fails unambiguously.

Neumann (1975, 1981) proposed that the timing of emergence in the intertidal midge *Clunio* is controlled by two internal rhythmic systems in individual animals: a circa-semilunar rhythm related to the timing of pupation, and a circadian rhythm that determines the daily timing of emergence. He thought that, as a combination of these internal timing systems, emergence occurs at the time of low tide around the full and new moons.

The primary features of the larval release rhythm of *Sesarma* are as follows: (1) tidal and semilunar components are involved at the same time, and both recur at semi-monthly intervals; (2) the synchronizing agents of tidal and semilunar rhythms are the same; (3) tidal rhythm can be entrained by a moonlight cycle without the 24.5-h component; and (4) the semilunar component is detectable when the tidal component is clearly seen. These facts suggest that the tidal and semilunar rhythms of *Sesarma* do not exist separately in each animal, but must be identical. If one agrees with this viewpoint, then the pattern of the semilunar rhythm can be accounted for by the coupled circadian oscillatory systems mentioned above. In this case, endogenous semi-monthly component superimposed on the circa-tidal rhythm must actually be a function of the β-oscillation. The driven β-oscillator itself possesses a fortnightly amplitude modulation, the level of which affects the timing of egg-production and larval release possibly mediated by endocrine systems.

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